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Nutritional and chemical composition of different life stages of *Tribolium castaneum* (Herbst)

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ABSTRACT

Tribolium castaneum can survive in extremely dry environments and be one of the major insect pests of broken and processed grains or other stored dried foods. Additionally, this species has demonstrated resistance to some classes of insecticides. The objective of this work was to evaluate the nutritional and chemical composition of *T. castaneum*, reared on maize flour, and compare protein, lipids and fatty acids profile, amino acids and mineral contents, of different developmental stages: larvae, pupae and adults. In general all stages were rich in protein (15.3% for larvae and 17.0% for adults, wet body weight).

Disparities among developmental stages regarding amino acids and fatty acids content were found. Essential amino acids were most abundant in *T. castaneum*; a different essential amino acid was prevalent on each developmental stage: larvae - valine; pupae - lysine; adults - histidine. *Tribolium castaneum* is rich in phosphorus, potassium and sulphur; larvae had a significantly higher content of phosphorus, potassium, sulphur and zinc, while adults showed significantly lower content of potassium and zinc. Larvae and pupae showed higher values of SFA (heptadecanoic and stearic acid) and PUFA (α-linolenic and linolenic acids), while MUFA showed the reverse tendency, with adults having the highest contents of hypogeic, palmitoleic and oleic acids. The nutritional differences among different developmental stages identified are due to specificities of each stage (level of chitinization, motility, energy requirements and food intake habits), as the diet offered to the insects was the same. This study can thus be considered a first step towards future directions of investigation, to a better understanding of this pest nutritional preferences and alternatives to achieve a more sustainable management of infested stored products.

1. Introduction

With the expected increase of the world population, the limited available land and the consequent increase in the demand for animal products, there will be an enormous need for resources, including food, to produce them. The fuel-feed-food competition is expected to further aggravate the situation. The search for new food resources of animal origin is essential and insects can be a good alternative source as novel-food.

Insects nutritional analyses shows that their composition is, on the overall, well balanced in terms of nutrients (Nowak et al., 2016; Costa et al., 2020). Generally, the main components of insects are protein and fat, and they also provide essential amino acids, unsaturated fatty acids and micronutrients (Belluco et al., 2013; Rumpold and Schlüter, 2015). Their nutritional profile may differ with insect species, but also with other characteristics, as the insect origin, developmental stage or diet (Finke and Oonincx, 2014; Gere et al., 2019).

Nutritional composition of insects has been analyzed, including not only insects classified as edible but also some insect pests (Rumpold and Schlüter, 2013; van Huis et al., 2013). The unique known study on chemical composition of stored product pests was made by Singh and Sinha (1977) studying carbohydrate, lipid, and protein contents in the

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developmental stages of *Sitophilus oryzae* (L.) and *S. granarius* (L.). They confirmed that adults of both species were rich in proteins and lipids, with values of 78.3–75.7% and 12.7–15.5% respectively (results as % of dry body weight). Both species are considered primary pests and conventional chemical control methods such as insecticides are commonly used to eradicate their populations.

With the commitment to achieve the objectives of sustainable development, and under a perspective of circular economy, one of the alternatives to reduce the impact of insect pest's presence, would be to reuse infested grains in animal feed or to use insect residues as organic fertilizer, instead of the use of insecticides to control them. Another alternative, considering that the insect pests would not be harmful to human health, would be the mechanical control, with the advantage of having a low environmental impact, and thus obtaining an additional source of animal product (van Huis et al., 2013; Patel et al., 2019). Some insect pests are considered edible, and are indeed used as food in some countries (e.g. Ramos-Elorduy, 1997; Cerritos and Cano-Santana, 2008). These initiatives would contribute to reducing waste and to a more sustainable pest management practices from an environmental, economic, and social perspectives.

The assessment of food preferences for stored product pests tends to focus on the nutritional and chemical characterization of the stored products along with the preference displayed by the insect (Mebarkia et al., 2009; Metwaly et al., 2015). The evaluation of the effects of different types of stored products on the nutritional and chemical profile of insects would be a valuable information to detect which changes may induce food preferences, making it possible not only to infer the different levels of resistance of stored products but also to identify possible physiological pathways that drive insects' food preferences.

The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is a polyphagous secondary pest of processed stored grains, being one of the most prevalent pests and having a worldwide distribution. This species has been recorded on 246 commodities (Hagstrum and Subramanyam, 2009), showing preference for flours and amylaceous by-products. It has also been reported that *T. castaneum* has developed resistance to some insecticides commonly used to control stored products insect pests such as phosphine, malathion, fenitrothion, and pirimiphos-methyl (Zettler et al., 1990; Zettler, 1991; Boyer et al., 2012; Opit et al., 2012; Gautam and Opit, 2015; Upadhyay et al., 2018) and to *Beauveria bassiana* because tenebrionid cuticular secretions have antifungal properties (Padin et al., 1997; Akbar et al., 2004; Lord, 2007; Pedrini et al., 2015). The adults of the red flour beetle produce two compounds methyl-1,4-benzoquinone and ethyl-1,4-benzoquinone and it is assumed that these compounds work as an external defense mechanism, killing microbes and deterring predators (Yezerski et al., 2007). El-Mofty et al. (1992) studied the impact of benzoquinones secreted by *T. castaneum* adults. For one year, the authors fed Swiss albino mice with three different substrates: flour infested with *T. castaneum,* cookies made from infested flour and 1,4-benzoquinone. They considered benzoquinone to be the cause of cancer incidence; however these animals were constantly fed for one year with the infested substrate. IARC (International Agency for Research on Cancer, 1999) considered that 1,4-benzoquinone is not classifiable as to its carcinogenicity to humans (Group 3) and Fardisi et al. (2017) consider that benzoquinone toxicity and carcinogenic effects need further investigations..

The Directive 2009/128/EC of the European Parliament and of the Council of October 21, 2009, approved on February 12, 2019, in Strasbourg (A8-0045/2019; Regulation (EU) 2015/2283), establishes a framework for action at the Community level for the sustainable use of pesticides. This Directive calls on the Commission and the Member States to place greater emphasis on investment and research into the development of precision in agriculture, based on a systemic approach combining innovative techniques and preventive measures aimed at minimizing pesticide dependence. Under both these points of view, the present work pretends to study the chemical and nutritional composition of *T. castaneum*.

The objective of this work is to evaluate the chemical composition of larvae, pupae, and adults of *T. castaneum,* reared on maize flour. This will allow the evaluation of the differences among the different developmental stages of *T. castaneum*, and also to contribute to the body of knowledge that may allow the investigation on *T. castaneum* feeding preferences, as well as a future evaluation of the re-usage of infested stored products for animal feed or as organic fertilizers, for example. New developments on pesticide alternatives, as the re-use of infested stored products for feed and resultant residues by insect activity as fertilizers, together with a good preventive program, taken stored products insect pests food preferences into account, should be integrated as new tools on stored product protection. As a first approach towards these future directions, the nutritional and chemical assessment of *T. castaneum* can be a step towards this understanding, as a tool that helps to achieve more sustainable management of the infested stored products.

2. Materials and methods

2.1. Insects rearing

Tribolium castaneum insects used in the trials were obtained from natural populations of the insect, with less than five years of rearing at the Entomology Laboratory of the Departamento de Ciências e Engenharia de Biossistemas (DCEB) of ISA, University of Lisbon. The cultures were maintained at 26 °C and 65–70% of relative humidity (RH) in a mixture of wheat flour and beer yeast (*Saccharomyces cerevisiae* Hansen) in a 95:5 proportion, according to Haines (1991).

For mass rearing of *T. castaneum* insects in maize flour, twenty-five glass flasks with 20 g of maize flour and 40 *T. castaneum* adults were maintained at 30 °C and 70% RH. After two weeks the adults were removed and the insects, from next generation, started to be collected to perform 50 g of each developmental stage: unsexed adults with less than two weeks, pupae with less than five days, and all stages of larvae. Larvae, and adults were separated from flour using a 500 μm mesh screen. For collecting pupae, flour was sieved through an 800 μm mesh. All individuals were removed with a soft-tipped tweezers.

All collected biological material was stored at −20 °C until further analysis. For each nutritional or chemical feature evaluated, three replicates of the same developmental stage were analyzed.

2.2. Nutritional analyses

2.2.1. Proximate analysis

2.2.1.1. Moisture content. The biological samples (initial weight of 3 g) were kept in the oven at 60 °C for approximately one week, until constant weight.

2.2.1.2. Ash content. Fresh biological samples with an initial weight of 2 g were placed in a muffle furnace at 550 °C. After 24 h the final weight of the samples was obtained and the ash content was determined.

2.2.1.3. Protein content. Nitrogen content (N) was determined according to the Kjeldahl method from 1 g of fresh biological material and crude protein content was obtained with a specific conversion factor for *Tenebrio molitor* L. (Coleoptera, Tenebrionidae), 5.41 (Boulos et al., 2020).

2.2.1.4. Lipids. Lipids were extracted from fresh samples of 1 g of biological material with ethyl ether in a Soxhlet device, according to a previously described method (NP 1972; IPQ, 2009). After solvent evaporation, fat residues were dried at 103 ± 2 °C until a constant weight was achieved.

2.2.1.5. Fibre content. Crude fibre determination was done according to the Weende method, using 1 g of dried biological material. Acid hydrolysis with 1.25% H_2SO_4 was performed for the extraction of sugars and starch, followed by alkaline hydrolysis with 1.25% NaOH, which removes proteins and some hemi-cellulose and lignin. After this, 150 mL of KOH of 0.2 mol/L concentration were added to the sample and boiled for 30 min. The sample was then washed with acetone in a Kitasato flask. The sample was dried in an oven at 103 °C and then in a muffle at 550 °C.

2.2.1.6. Nitrogen free extract. Nitrogen free extract (NFE) represents digestible carbohydrates, such as sugars and starches, among others and was calculated by difference: NFE = 100 - (moisture content % + crude protein % + crude fat % + crude fibre % + ash %) (AOAC, 1990).

2.2.2. Fatty acids

Fatty acid methyl esters (FAME) were prepared from lyophilized samples. The transesterification was done by acid catalysis according to a previously described method (Bandarra et al., 1997). FAME chromatographic analysis was done at 250 °C, using a Varian Star CP-3800 Gas Chromatograph equipped with an automatic sampler with a split injector and a flame ionization detector. The separation was done in a capillary column with polyethylene glycol stationary phase: Agilent J&W GC Column DB-Wax length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm, using helium as the carrier gas and following a temperature program starting at 180 °C and increasing temperature to 200 °C at 4 °C/min, remaining 10 min at 200 °C, and, after this, heating to 210 °C at the same rate, and staying at this temperature for 14.5 min.

FAME were identified by comparing their retention time with those of the Sigma – Aldrich standard (PUFA-3, Menhaden oil). The limit of detection (LOD) was 1 mg/100 g. The results were calculated as a percentage of total fatty acids based on peak areas and the results (mg/100 g) were obtained using the internal standard method (10 mg/mL of heneicosane acid, 21: 0).

2.2.3. Amino acids profile

Fresh biological samples were weighed (0.25 g), ground in a mortar and hydrolyzed for 24 h at 110 °C in an oven (Heraeus, Hanau, Germany) with 5 mL of 6 M HCl in sealed pyrex test tubes (Thomas Scientific, Sheldon, USA). The samples were then filtered, and 200 μL of the amino acids extract was mixed with 800 μL of a mixture of 25 mg orthophthalaldehyde (OPA), 0.5 mL methanol, 5 mL borate buffer 0.7 M and 25 μL 2-mercaptoethanol. After vortexed for 1 min, the reaction mixture was immediately injected into the HPLC system.

Amino acids were identified and quantified using a Waters HPLC system consisting of an Alliance 2695 separation module (Waters, Milford, USA) and a fluorescence detector (Waters 2475 MultiFluorescence, Waters, Milford, MA). Chromatographic separation was performed in a reverse-phase column (Spherisorb ODS2 C18, 250 × 4.6 mm, 5 μm, Waters, Milford, USA), at room temperature, using an elution gradient with a mixture of 2 solvents. Solvent A - 0.1 M sodium acetate:methanol: tetrahydrofuran (5:90:5) and solvent B - methanol. The gradient changed from 0 to 25% of solvent B in 20 min and from 25 to 100% in 30 min at a flow rate of 1.0 mL/min. The column was equilibrated during 10 min before the next analysis. The separation was monitored using a fluorescence detector at 338 nm (excitation) and 425 nm (emission). The 18 amino acids (aspartic acid, asparagine, glutamic acid, glutamine, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine and lysine, Sigma Aldrich, St. Louis, USA) were identified by comparison with the retention time of standards and their quantification was based on the external standard technique.

2.2.4. Mineral content

Fresh biological samples were dried at 60 °C and weighed (0.5 g). The samples were ground in a mortar and weighted into a Teflon tube to which 3 mL of concentrated nitric acid and 10 mL of concentrated hydrochloric acid were added. After 24 h, 1 mL of hydrogen peroxide was added, and the tube was placed in a digester with a heating cycle to 95 °C that lasts 1 h and remains at 95 °C for another hour. After removal from the digester, the samples were cooled, transferred to a 25 mL volumetric flask and the volume was adjusted to 25 mL with distilled water. The quantification of phosphorus (P), potassium (K), sulphur (S), sodium (Na), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) was made by ICP-AES (*Inductively coupled plasma atomic emission spectroscopy*, Thermo Scientific iCAP 7200). Appropriate standards were prepared from a stock solution (100 mg/L) containing the analyzed elements (SCP Science, PlasmaQual S22). Results were expressed as mg/kg dry mass.

2.2.5. Statistical analyses

The nutritional profile of the three developmental stages of *T. castaneum* were compared in order to detect significant differences among them ($p < 0.05$). For this, assumptions were tested with the Bartlett test for homoscedasticity and the Shapiro-Wilk test for normal distribution of residues. Then, the data obtained were submitted to an analysis of variance (ANOVA) and when the result was considered to be significant (for $p < 0.05$), Tukey's honestly significant difference test (HSD) was done. All these analyses were performed with RStudio (RStudio Team, 2020) and R-3.1.2.

3. Results

Proximate compositions (crude protein, crude fat, crude fibre, ash and nitrogen free extract) were determined for larvae, pupae and adults of *T. castaneum* (Table 1). The results for the water content of each developmental stage of *T. castaneum* demonstrate that, as expected, the adults have less water. Adults had significantly higher fibre content, as well as crude protein and nitrogen free extract. Pupae had significantly lower ash content.

The *T. castaneum* life stages analyzed were rich in phosphorus, potassium and sulphur (Table 2). The iron, copper, sodium, and calcium contents are similar among different developmental stages. On the contrary, larvae had a significantly higher content of phosphorus, potassium, sulphur and zinc. Adults showed significantly lower content of potassium and zinc, comparatively to larvae and pupae.

Relatively to amino acid content, larvae have significantly higher values of aspartic acid and valine than adults (Table 3). Pupae have lower values of glutamine, comparing to adults. The most abundant amino acids were the same for the different developmental stages:

Table 1
Proximate compositions, including average values for: Water content (%), crude protein (%), crude fat (%), crude fibre (%; no replicates), ash (%) and nitrogen free extract (%) of larvae, pupae and adults of *T. castaneum*. Three replicates were done for each category (df = 2), except for fibre, with two replicates (df = 1). Different letters following the values in the same column indicate significantly different ($p < 0.05$) values.

Proximate analysis	Larvae	Pupae	Adults	F value	P value
Water content (%)	56.34 ± 0.62 a	54.33 ± 0.06 a	46.87 ± 1.56 b	74.73	<0.001
Ash content (%)	1.15 ± 0.00 a	1.00 ± 0.05 b	1.16 ± 0.08 a	9.10	0.015
Crude fat (%)	6.36 ± 1.01 a	6.41 ± 0.89 a	7.24 ± 0.17 a	1.19	0.368
Crude fibre (%)	1.88 ± 0.15 a	1.99 ± 0.02 a	15.13 ± 0.10 b	336.16	<0.001
Crude protein (%)	15.30 ± 0.18 a	15.56 ± 0.23 a	16.97 ± 0.87 b	16.78	0.018
Nitrogen free extract (%)	18.95 ± 1.05 a	20.71 ± 0.99 a	12.65 ± 1.46 b	8.56	0.018

Table 2
Average mineral content (mg/100 g of dry mass) of larvae, pupae and adults of *T. castaneum* reared in maize flour. Three replicates were done for each category (df = 2 for all the analyses). Different letters following the values in the same row indicate significantly different ($p < 0.05$) values.

Minerals	Larvae	Pupae	Adult	F value	P value
Phosphorus	553.07 ± 13.94 a	434.92 ± 12.30 b	426.02 ± 20.72 b	58.43	<0.001
Potassium	434.16 ± 5.67 a	368.27 ± 7.44 b	319.94 ± 18.57 c	68.40	<0.001
Sulphur	280.05 ± 9.43 a	252.66 ± 9.80 b	258.02 ± 5.90 b	8.62	0.017
Sodium	92.73 ± 3.53 a	85.37 ± 2.34 a	89.67 ± 5.65 a	2.46	0.166
Magnesium	71.68 ± 5.28 a	63.28 ± 0.86 ab	57.89 ± 2.57 b	12.31	<0.001
Calcium	19.28 ± 2.13 a	20.62 ± 1.66 a	22.35 ± 0.92 a	2.54	0.159
Zinc	11.30 ± 0.11 a	6.42 ± 0.35 b	7.16 ± 0.26 c	12.31	<0.001
Iron	6.98 ± 0.56 a	8.22 ± 1.14 a	6.35 ± 0.70 a	3.87	0.083
Copper	0.98 ± 0.12 a	1.01 ± 0.58 a	1.03 ± 0.69 a	0.50	0.628
Manganese	0.49 ± 0.03 a	0.53 ± 0.03 ab	0.57 ± 0.01 b	7.72	0.022

Table 3
Average amino acid content (mg/100 g of dry mass) of larvae, pupae and adults of *T. castaneum* reared in maize flour. Three replicates were done for each category (df = 2 for all the analyses). Different letters following the values in the same row indicate significantly different ($p < 0.05$) values.

Amino acids	Larvae	Pupae	Adult	F value	P value
Lysine[a]	149.44 ± 16.33 a	185.20 ± 12.34 a	169.90 ± 17.57 a	3.98	0.079
Histidine[a]	147.86 ± 43.23 a	149.04 ± 30.94 a	205.35 ± 52.76 a	1.73	0.255
Valine[a]	129.43 ± 14.71 a	115.26 ± 27.68 ab	68.38 ± 16.18 b	7.38	0.024
Threonine[a]	22.28 ± 9.15 a	28.61 ± 7.08 a	26.65 ± 12.23 a	0.33	0.728
Leucine[a]	26.44 ± 2.48 a	21.37 ± 1.98 a	25.65 ± 2.51 a	4.07	0.076
Methionine[a]	14.77 ± 2.03 a	12.61 ± 2.38 a	13.88 ± 2.33 a	0.69	0.536
Isoleucine[a]	8.70 ± 2.66 a	8.35 ± 0.77 a	12.28 ± 2.08 a	3.55	0.096
Phenylalanine[a]	3.29 ± 1.83 a	4.14 ± 0.63 a	6.07 ± 1.81 a	2.60	0.153
Tyrosine	79.86 ± 24.40 a	56.58 ± 26.05 a	62.35 ± 24.45 a	0.71	0.530
Arginine	79.14 ± 61.29 a	48.02 ± 5.57 a	58.42 ± 32.28 a	0.47	0.647
Serine	55.23 ± 5.91 a	61.65 ± 7.22 a	55.08 ± 8.54 a	0.79	0.495
Glutamine	40.61 ± 8.93 ab	36.66 ± 7.06 a	62.51 ± 13.04 b	5.83	0.039
Alanine	51.57 ± 31.76 a	57.49 ± 27.86 a	19.94 ± 13.45 a	1.86	0.234
Glycine	19.32 ± 7.66 a	29.90 ± 8.13 a	14.77 ± 5.78 a	3.42	0.102
Glutamic acid	20.62 ± 1.69 a	18.66 ± 1.24 a	17.52 ± 1.19 a	3.82	0.085
Ornithine	14.35 ± 5.81 a	16.28 ± 2.39 a	17.31 ± 8.57 a	0.18	0.840
Asparagine	15.83 ± 2.89 a	15.98 ± 3.69 a	13.44 ± 4.32 a	0.45	0.657
Aspartic acid	13.33 ± 1.80 a	11.79 ± 0.55 ab	10.00 ± 0.65 b	6.28	0.034

[a] essential amino acids.

histidine for adults, lysine for pupae and valine for larvae, all of them belonging to the groups of essential amino acids.

Regarding saturated fatty acids (SFA), adults had a significantly lower amount of heptadecanoic acid (C17:0), and larvae had a significantly higher amount of stearic acid (C18:0) (Table 4). All three developmental stages were considered to have statistical differences relatively to total polyunsaturated fatty acids (PUFA), with larvae concentrating the largest amount of PUFA and adults the smallest and this was verified specifically in linoleic acid (C18:2n-6). For α-linolenic acid (C18:3n3) pupae had nearly two times the amount of this fatty acid comparatively to adults. Adults had significantly higher values of total monounsaturated fatty acids (MUFA), comparatively to larvae and

Table 4
Average fatty acids content (% of dry mass) of larvae, pupae and adults of *T. castaneum* reared in maize flour. SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids. Three replicates were done for each category (df = 2 for all the analyses, except for SFA C22:0 and PUFA C20:2n-6 (df = 1 for both)). Rows without letters and with an asterisk indicate that only one value per developmental stage was available, precluding the statistical analysis. Different letters following the values in the same row indicate significantly different ($p < 0.05$) values.

Fatty acids		Larvae	Pupae	Adults	F value	P value
SFA	C14:0	0.47 ± 0.03 a	0.76 ± 0.31 a	0.57 ± 0.11 a	1.27	0.358
	C16:0	28.11 ± 2.55 a	30.02 ± 4.70 a	31.12 ± 1.50 a	0.68	0.542
	C17:0 isobr			0.10*		
	C17:0	0.20 ± 0.00 a	0.22 ± 0.01 a	0.14* b	77.4	0.013
	C18:0	10.30 ± 0.14 a	9.28 ± 0.50 b	6.04 ± 0.23 c	222.70	<0.001
	C20:0	0.21 ± 0.01 a	0.23 ± 0.04 a	0.16* a	4.42	0.185
	C22:0	0.17 ± 0.01 a	0.25 ± 0.04 a		7.76	0.108
MUFA	C16:1n-9	0.36 ± 0.03 a	0.50 ± 0.04 b	0.42 ± 0.04 ab	7.88	0.041
	C16:1n-7	0.20 ± 0.03 a	0.21 ± 0.00 a	0.64 ± 0.06 b	81.69	<0.001
	C18:1n-7	32.06 ± 0.80 a	30.35 ± 0.64 a	38.27 ± 0.74 b	97.44	<0.001
	C20:1n-11	0.08*	0.15*			
	C20:1n-9	0.06*				
PUFA	C16:3n-3			0.11*		
	C18:2n-6	21.18 ± 0.58 a	17.52 ± 0.47 b	15.47 ± 0.07 c	134.80	<0.001
	C18:3n-3	0.22 ± 0.01 ab	0.29 ± 0.02 a	0.14* b	29.16	0.033
	C20:3n-3	0.08*				
	C20:2n-6	0.22 ± 0.22 a	0.27 ± 0.21 a		0.06	0.823
TOTAL	FAME	93.08 ± 2.64 a	89.28 ± 2.86 a	92.75 ± 1.18 a	2.42	0.170
	NI	6.92 ± 2.64 a	10.72 ± 2.86 a	7.25 ± 1.18 a	2.42	0.170
	X1	4.97 ± 0.77	8.15 ± 1.56	5.15 ± 0.92		
	SFA	39.11 ± 1.97 a	40.51 ± 4.31 a	37.86 ± 1.21 a	0.66	0.551
	MUFA	32.48 ± 0.44 a	30.87 ± 1.05 a	39.34 ± 0.82 b	93.02	<0.001
	PUFA	21.49 ± 0.57 a	17.89 ± 0.44 b	15.55 ± 0.13 c	149.80	<0.001
	n-3	0.17 ± 0.15 a	0.19 ± 0.17 a	0.08 ± 0.14 a	0.41	0.683
	n-6	21.32 ± 0.65 a	17.70 ± 0.34 b	15.47 ± 0.07 c	145.2	<0.001
	Ratio n-3/n-6	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.28	0.767
	SFA + PUFA + MUFA	93.08 ± 2.64 a	89.28 ± 2.86 a	92.75 ± 1.18 a	2.42	0.170

pupae, and specifically palmitoleic acid (C16:1n-7) (three times higher) and oleic acid (C18:1n-7). The larva was considered to have significantly lower values of hypogeic acid (C16:1n-9) comparing to pupae. The polyunsaturated to saturated fatty acid ratio was 1.4 for larvae and adults and 1.2 for pupae.

4. Discussion

Tribolium castaneum has Malpighian tubules between the midgut and hindgut to absorb water and because it has hydrosensitive sensilla to detect water, this insect is physiologically well adapted to the very low moisture content of the stored product (Murdock et al., 2012), especially the adult stage. This may be related to the development of the chitinous exoskeleton (Li et al., 2016; Drevinskas et al., 2017; Hamdi et al., 2018). The ash content of *T. castaneum* was high (>10%), and although pupae had lower values, the total mineral content was not the lowest. This discrepancy may be linked to the loss of volatile minerals due to the high temperatures used on the ash determination process.

Tribolium castaneum developmental stages showed to be equally rich in phosphorus, as the majority of nutritionally evaluated insects (Rumpold and Schlüter, 2015), as well as in potassium and sulphur. Zinc, iron and copper contents were higher on larvae stages. The quantity of sodium detected was low for all developmental stages.

Tribolium castaneum, feeding on maize flour, has a rich protein content, although lower than *T. molitor* (Ravzanaadii et al., 2012; Rumpold and Schlüter, 2013). Although adults presented a higher content of protein, it was not significantly different when compared with the results of the other developmental stages. Accordingly, disparities among different developmental stages of *T. molitor* also exist with a clear prevalence of higher protein content in the adults (Nowak et al., 2016), and lower in larvae (Rumpold and Schlüter, 2013).

Essential amino acids were the most abundant in *T. castaneum* and interestingly, each state of development had predominantly a different essential amino acid: larvae - valine; pupae - lysine; and adults - histidine. The significantly lower content of aspartic acid in the insect adults may be linked to higher levels of threonine and lysine, which are known to have a down-regulatory effect on aspartic acid synthesis enzymes (Nascimento Filho et al., 2021). However, as pupae content of these two amino acids, as well aspartic acid content, was higher when compared with adults, there must be another regulatory pathway interfering. Lower glutamine content in the pupae stage is probably linked to the higher content in alanine and glycine, which may have a feedback inhibition effect on glutamine biosynthesis (Tate and Meister, 1971).

Cereal based diets have deficiencies in providing essential amino acids. For example, maize flour nutritive value is known for being deficient in proteins, and amino acids are part of its limiting nutrients, namely lysine and tryptophan (FAO and INPhO, 1993). The enrichment of bakery goods with insect flour has already proven to be positive towards the content and bioavailability of essential amino acids, for example (González et al., 2019; Oliveira et al., 2017; Osimani et al., 2018).

The larval stage has a larger fat body to store energy, usually, in the form of fatty acids and glucose, that is used for several functions, for example, metamorphosis, new adult emergence, adult flight, embriogenesis or immune responses (Arrese and Soulages, 2010). In the present work, there were no significant differences in fat content and only pupae had significantly lower ash content. The differences denoted in this study may be caused by the physiological arrangement of this insect, where larvae start accumulating reserves to pass through metamorphosis (pupae) and then survive as adults. As adults also feed on maize flour, the supply of energy reserves may be used to improve their status, especially for mating and oviposition. Generally, larvae and pupae showed higher values of SFA (heptadecanoic and stearic acid) and PUFA (α-linolenic and linolenic acids), which may be explained by the fat body reserves, which then start to be depleted in the adult stage, since flight and reproduction functions are only active in this developmental stage and are energy demanding activities. Interestingly, MUFA showed the reverse tendency, with adults having the highest contents of hypogeic, palmitoleic and oleic acids. A hypothesis for this would be the continuation of the storage of energy within the fat body in the adult stage, as this insect is long living as an adult (Mahroof and Hagstrum, 2012). It is indeed known that the fatty acid and acetate incorporated into the fat body is dependent on the developmental stage of the insect, as well as on its diet (Arrese and Soulages, 2010). The amount of saturated fatty acids (SFA) was lower than the amount of unsaturated fatty acids found in all *T. castaneum* life stages. The most abundant fatty acids, per category, were palmitic acid (SFA), vaccenic acid (MUFA) and linoleic acid (PUFA). These tendencies were observed in all life stages, probably being a trend in *T. castaneum* species, when fed on maize flour. The ratio between stearic acid and palmitic acid (C18:0/C16:0) may induce worse lipid profile, insulin resistance, and alter mRNA expression, being advised to be taken into account for evaluating nutritional resources (Wang et al., 2020). In *T. castaneum* it was observed that this ratio was small, as palmitic acid is more abundant than stearic acid. The polyunsaturated to saturated fatty acid ratio is a significant marker of lipid composition, and should be close to 1 (Paul et al., 2017), as observed for *T. castaneum* in this work. Thus, regarding the fatty acid profile of *T. castaneum*, this insect could be considered a well-balanced nutritional resource. The fatty acid content may vary not only with species, developmental stage, and rearing location, but also with insect's diet (Nowak et al., 2016; Paul et al., 2017). Additionally, changes in *T. castaneum* diet composition may interfere with the insect development and nutritional composition (Fabres et al., 2014). In the future, it would be interesting to evaluate the effect of different diets on the fatty acid profile of *T. castaneum*, ideally comparing different stored products.

From a circular economy perspective, the increase in human population and the consequent need to increase animal and feed production, could lead to the conclusion that the reusage of infested grains and derivatives for animal feed and insect residues, as fertilizers, would be advisable. There is, however, the need for further characterization of *T. castaneum* infestation effects on stored products regarding possible hazards associated with allergens and production of defensive substances, as benzoquinones, as nutritionally these insects demonstrated to have a well-balanced profile. Overall, the tolerance of putatively small populations of *T. castaneum* in maize flour could result in a positive outcome from the nutritional point of view as these insects are rich in essential amino acids and minerals and unsaturated fatty acids, possibly acting as a fortification of maize flour. This chemical assessment of *T. castaneum* can thus contribute to find innovative and more sustainable solutions for the management of stored products.

Author statement

Sónia Duarte: Formal analysis, Visualization, Writing – original draft, João Limão: Methodology, Investigation, Graça Barros: Methodology, Resources. Narcisa Maria Bandarra: Methodology, Resources, Investigation. Luísa Cristina Roseiro: Methodology, Resources, Investigation. Helena Gonçalves: Methodology, Investigation. Luísa Louro Martins: Conceptualization, Methodology, Resources, Investigation, Validation, Writing – review & editing. Miguel Mourato: Conceptualization, Methodology, Resources, Investigation, Validation, Writing – review & editing. Maria Otília Carvalho: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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